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Behavioral Batesian Mimicry Involving Intraspecific Polymorphism in the Butterfly *Papilio polytes*

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Batesian mimics gain protection from predation by their similarity to distasteful models. In butterflies, it has been thought that distasteful species and Batesian mimics fly slowly and in a straight line, but few studies have demonstrated their behavioral similarity, and no studies have been conducted on behavioral mimicry involving Batesian intraspecific polymorphism. Here, we compared the wing stroke among various butterflies: palatable non-mimetic *Papilio xuthus*, unpalatable *Pachliopta aristolochiae*, and palatable polymorphic *Papilio polytes* (*cyrus* form, non-mimetic females; *polytes* form, Batesian mimetic females) to clarify whether the wing stroke of unpalatable butterflies is different from that of palatable species, whether that of the non-mimetic females of *Pap. polytes* is different from the mimetic females, and whether that of the mimetic females resembles that of the model. We found that the minimum positional angle (ϕ_{min}) of *Pach. aristolochiae* and mimetic females of *Pap. polytes* was significantly larger than that of *Pap. xuthus* and non-mimetic females. We did not detect significant differences between that of *Pach. aristolochiae* and mimetic females of *Pap. polytes*. These results show that ϕ_{min} differed between the mimicry group and palatable butterflies. In addition, the wingbeat frequency (WBF) of *Pach. aristolochiae* and mimetic females tended to differ from that of *Pap. xuthus* and non-mimetic females. This result suggests that there may be convergence of WBF in Batesian mimicry groups, as in the case of Müllerian mimicry groups, and serves as the first evidence of behavioral mimicry in Batesian intraspecific polymorphism.

Key words: behavioral mimicry, flight behavior, Batesian mimicry, butterfly, intraspecific polymorphism, *Papilio polytes*, *Pachliopta aristolochiae*, wing motion

INTRODUCTION

Many distasteful animals possess conspicuous coloration. Experimental and computer simulation studies have demonstrated that predators learn to avoid conspicuous distasteful prey more readily than cryptic prey, and thus the coloration is considered to have a warning function (Gittleman and Harvey, 1980; Yachi and Higashi, 1998). Indeed, such distasteful animals have increased protection from predators resulting from their coloration in nature (Benson, 1972; Edmunds, 1974; Ruxton et al., 2004).

Many distasteful animals that have warning coloration exhibit sluggish movements (Edmunds, 1974; Pasteels et al., 1983; Hatle and Faragher, 1998). In butterflies, the flight behavior of unpalatable species differs from that of palatable ones: butterflies that do not have defensive chemicals fly erratically. This erratic movement may make it difficult for predators to predict the flight path, reducing the frequency of successful attacks by the predators. By contrast, butterflies that have defensive chemicals in their bodies fly regularly. This flight pattern may increase the conspicuousness of their warning coloration to enhance learning, avoid confusion with palatable butterflies, decrease the chance of mistaking unpalatable species as palatable species, and decrease the chance of attacks by potential predators (Chai and Srygley, 1990). Moreover, because palatable butterflies have high maneuverability, they may be able to escape from birds' attacks more successfully (Chai and Srygley, 1990; Srygley and Dudley, 1993). This difference in flight patterns between palatable and unpalatable species is correlated with differences in morphological, physiological, and other behavioral traits (Srygley and Chai, 1990a, b; Marden and Chai, 1991; Srygley and Dudley, 1993; Srygley, 1994).

Batesian mimic butterflies, which are palatable species that have coloration similar to unpalatable models, also gain protective effects from their coloration (Brower, 1958a, b, c; Uesugi, 1996). It has been thought that Batesian mimic butterflies mimic not only the coloration but also the flight behavior of their models to enhance the protective effects. Recently, Srygley (2004) reported that Batesian mimics and their models perform wingbeats with slow angular velocity compared to palatable species to enhance the color signal. However, the flight behavior of Batesian mimics is still poorly investigated, and skepticism remains over whether it exists (Brower, 1995).

Papilio polytes is a female-limited Batesian mimic butterfly. In addition, females of this butterfly show polymorphic

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coloration. Non-mimetic females (*Pap. polytes* form *cyrus*) resemble conspecific males, whereas mimetic females (*Pap. polytes* form *polytes*) resemble an unpalatable sympatric toxic butterfly, *Pachliopta aristolochiae* (Euw et al., 1968), and thus are thought to be a Batesian mimic of the latter species (Uesugi, 1991, 1996; Ohsaki, 1995). This polymorphic species provides a good opportunity to examine the co-occurrence of behavioral and coloration mimicry. Here, we made the following three predictions: (1) the flight behavior of *Pach. aristolochiae* is different from that of *Pap. xuthus* (palatable control), (2) the flight behavior of non-mimetic females is similar to that of the palatable species, and (3) the flight behavior of mimetic females is similar to that of the unpalatable species. To test these predictions, we video-recorded flight behavior and analyzed the video images.

MATERIAL AND METHODS

Animals

We used three species of Papilioninae, *Papilio polytes* (Papilionini) (Fig. 1A, B), *Pachliopta aristolochiae* (Troidini) (Fig. 1D), and *Papilio xuthus* (Papilionini) (Fig. 1C). *Papilio polytes* is a palatable swallowtail butterfly commonly found throughout the Oriental tropics. Males of this butterfly are monomorphic, whereas females are polymorphic. *Pachliopta aristolochiae* is an unpalatable toxic butterfly also found throughout the Oriental tropics. It has red warning spots on the hindwings. Poisonous substances are absorbed from the food plant by the larva and stored by the organism during pupation and metamorphosis (Euw et al., 1968). *Papilio xuthus*, which is not known to be involved in mimicry either as a model or as a mimic, is a swallowtail butterfly found throughout temperate East Asia. *Papilio polytes* and *Papilio xuthus* use species in Rutaceae as host plants, whereas *Pachliopta aristolochiae* uses species in Aristolochiaceae.

Adults of *Pap. polytes* and *Pach. aristolochiae* were collected on Ishigaki Island, Okinawa, Japan (124°8'E and 24°26'N), and were brought to Mino Park Insectary, Osaka, Japan (135°28' E and 34°50' N). Adults of *Pap. xuthus* were brought to the Insectary from Osaka Prefecture. Eggs of these butterflies were collected in the Insectary and were raised in a temperature-controlled room at 23°C. Butterflies that emerged from pupae were released into the Insectary. We performed behavioral observations on butterflies kept in the Insectary for at least one generation.

Wing stroke records

From May 2007 to April 2008 (from 10 am to 4 pm), We recorded the flight behavior of butterflies with a hand-held high-speed video camera (250 images per second; NAC model ST-549-J; recorder, HSV-500 C3) while they were flying freely in the Insectary. We waited for butterflies to fly to the same open place in the Insectary and recorded their flight. After recording the flight behavior of butterflies, we captured them and immediately measured ambient temperature (range, 21.1–33.6°C), and their wing length (left forewing, to the nearest 0.01 mm). All data were collected only once per individual.

Analysis of images

For the following analyses, we used images continuously recorded for more than one second (more than 8 wingbeats). Gliding flight was excluded from the analyses. We measured five variables from the images: wingbeat frequency (WBF), maximum positional angle (ϕ_{max}), minimum positional angle (ϕ_{min}), stroke amplitude (Φ), and angular velocity. WBF (Hz) was calculated as the mean number of wingbeats per second. ϕ_{max} and ϕ_{min} refer to the angles of the wing tip position in the stroke plane at the top and bottom of the half-stroke, respectively (Dudley, 2000). Because it is thought that forewings are more important than hindwings for butterfly flight, and hindwings are too small to measure directly from images, we used forewings in measuring these variables.

The wing positional angle (ϕ_{max}, ϕ_{min}) was defined as 0° when horizontal, positive when above horizontal, and negative when below horizontal. $\phi_{max,ind}$ was defined as the mean value of ϕ_{max} in one successive flight of a given individual, and mean ϕ_{max} was defined as the mean value of $\phi_{max,ind}$ for each species and morph. $\phi_{min,ind}$ and mean ϕ_{min} are corresponding values for ϕ_{min}. Φ is the angular extent of motion in the stroke plane, and was calculated as $\phi_{max} - \phi_{min}$. Mean Φ was defined as the mean value of Φ for each species/morph.

The angular velocity of a wing stroke was calculated as the total wing stroke angle ($\phi_{max} - 2 \times \phi_{min} + \phi_{max}$ of the next wing stroke) divided by the duration of a cycle of the wingbeat. Angular velocity was then averaged for one successive flight for a given individual. Mean angular velocity was defined as the mean value of angular velocity for each species/morph. The wing positional angle (mean ϕ_{max}, mean ϕ_{min}) was estimated visually. The error with this method was determined to be 4.02 ± 3.6°

Fig 1. Females of three butterfly species/morphs. **(A)** *Papilio polytes*, *cyrus* form (non-mimetic). (B) *Papilio polytes*, *polytes* form (mimetic). **(C)** *Papilio xuthus*. **(D)** *Pachliopta aristolochiae*.

(mean ± SD, n = 126) by a simulation test in which one observer, who was not informed of the wing positional angle of a model butterfly determined at various angles by another observer, estimated it visually.

Statistical analysis

For each sex/morph, we used one-way ANOVA (StatView 5.0) to test for differences among species/morphs in WBF, mean ϕ_{max}, mean ϕ_{min}, mean Φ, and mean angular velocity separately. We then conducted multiple comparisons (Bonferroni/Dunn, StatView 5.0) for variables for which we found significant differences. The significance level for statistical tests was set at $P = 0.05$.

RESULTS

In males, statistically significant effects of species were found for WBF (df = 2, 29, F = 9.615, P = 0.0006), ϕ_{max} (df = 2, 29, F = 3.552, P = 0.0417), ϕ_{min} (df = 2, 29, F = 6.140, P = 0.0060), and Φ (df = 2, 29, F = 6.293, P = 0.0054) (Fig. 2A–C). No significant effects of species were found for mean angular velocity (df = 2, 29, F = 1.182, P = 0.3210) (Table 1). In females, significant effects of species/morphs were found for ϕ_{min} (df = 3, 39, F = 5.959, P = 0.0019) and Φ (df = 3, 39, F = 3.515, P = 0.0239) (Fig. 2D, E), but not for WBF (df = 3, 39, F = 1.647, P = 0.1942), ϕ_{max} (df = 3, 39, F = 1.273, P = 0.2972) (Fig. 2F), or mean angular velocity (df = 3, 39, F = 2.102, P = 0.1156) (Table 1). There was no significant difference in wing length between non-mimetic and mimetic females of *Pap. polytes* (t-test; T value = 0.333, $P = 0.743$).

In males, Φ was significantly larger for palatable butterflies than for unpalatable species (Fig. 2A), and ϕ_{min} was significantly smaller for palatable species than for unpalatable species (Fig. 2B). Also for WBF, there were significant differences between unpalatable and palatable species (*Pap. xuthus* vs *Pach. aristolochiae*, P < 0.0005; *Pap. polytes* vs *Pach. aristolochiae*, P < 0.005; *Pap. xuthus* vs *Pap. polytes*, P = 0.3998) (Table 1). For ϕ_{max}, a significant difference was detected only between *Pap. xuthus* and *Pach. aristolochiae*, while the difference between *Pap. polytes* and *Pach. aristolochiae* fell short of significance (P = 0.0514) (Fig. 2C).

In females, a significant difference was found for Φ only between *Pap. xuthus* and *Pach. aristolochiae* (Fig. 2D). ϕ_{min} of non-mimic species/morphs was significantly smaller than for the mimicry group (Fig. 2E).

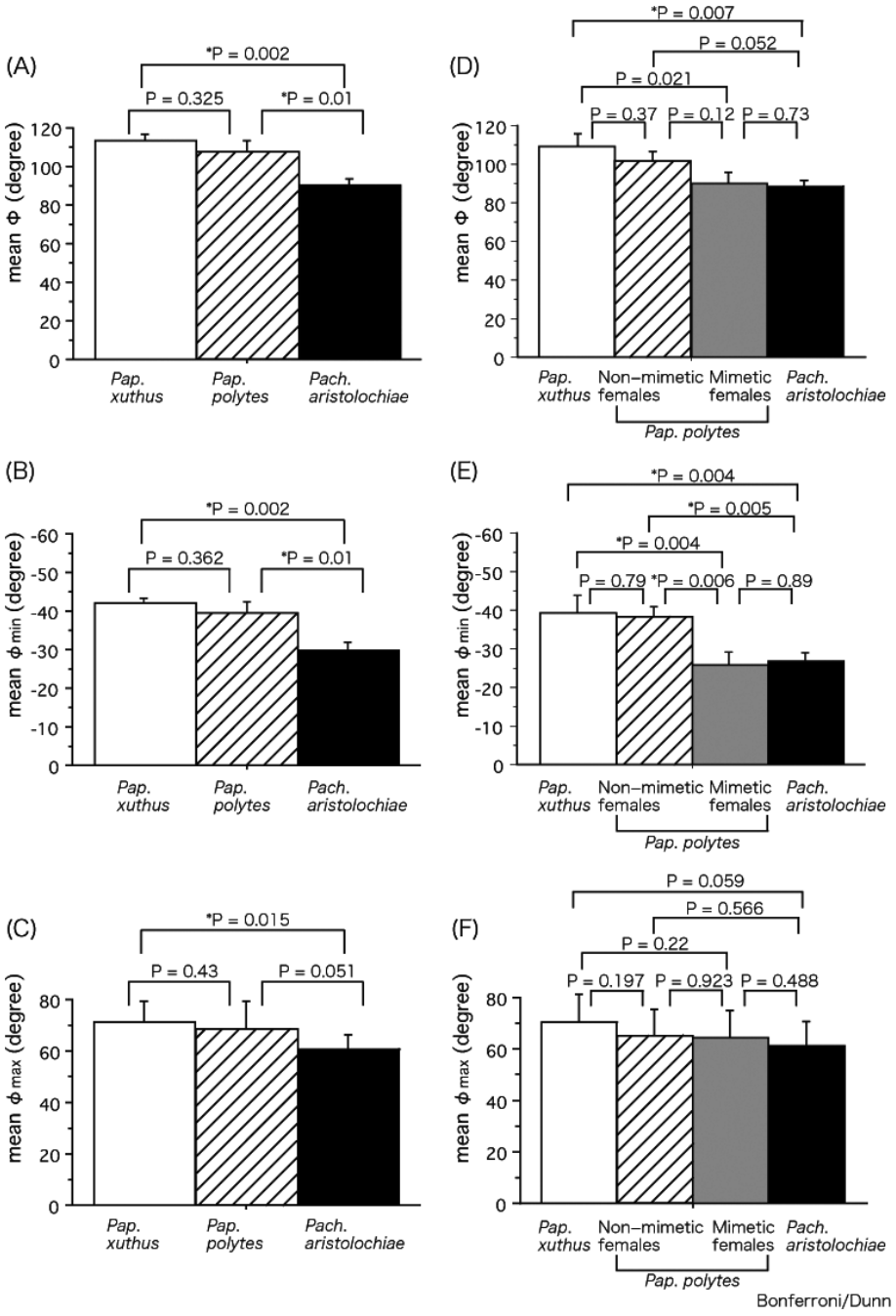

Fig 2. Comparisons of mean stroke amplitude (mean Φ) **(A, D)**, mean minimum positional angle (mean ϕ_{min}) **(B, E)**, and mean maximum positional angle (mean ϕ_{max} **(C, F)** among butterfly species/morphs. Bars indicate one SE. *Papilio xuthus*, a palatable non-mimetic species; *Papilio polytes*, a palatable species in which males are non-mimetic and females are polymorphic (non-mimetic and Batesian mimetic forms); *Pachliopta aristolochiae*, an unpalatable model species. Left **(A–C)**, males; right **(D–F)**, females. Significance levels are based on the Bonferroni correction (*P = 0.0167 and *P = 0.0083 for males and females, respectively).

DISCUSSION

The results support our predictions on locomotor mimicry in butterflies showing Batesian coloration mimicry. In particular, our results provide the first evidence showing

Table 1. Values of morphological and kinematic variables for three butterfly species (an unpalatable model species, a palatable polymorphic species, and a palatable control species).

Species/form	Group	n	Wing length (mm)	WBF (Hz)	Angular velocity (degree/msec)
Male					
Papilio xuthus	control	10	45 ± 3	10.3 ± 0.7	2.36 ± 0.2
Papilio polytes	non-mimic	14	41 ± 3	10.5 ± 0.9	2.31 ± 0.4
Pachliopta aristolochiae	model	8	45 ± 4	11.8 ± 0.5	2.14 ± 0.2
Female					
Papilio xuthus	control	8	48 ± 3	10.8 ± 0.8	2.44 ± 0.5
Pap. polytes f. *cyrus*	non-mimic	10	44 ± 4	10.8 ± 1.2	2.20 ± 0.3
Pap. polytes f. *polytes*	mimic	11	45 ± 3	11.3 ± 0.7	2.08 ± 0.5
Pach. aristolochiae	model	14	47 ± 2	11.4 ± 0.4	2.05 ± 0.3

N, sample size; WBF, wing beat frequency. Means ± SD are shown.

divergence in a flight behavior in butterflies with Batesian intraspecific polymorphism. A clear difference between mimetic and non-mimetic females was found in ϕ_{min}: mimetic females showed a larger ϕ_{min}, which was similar to that of their model. One possible function of a larger ϕ_{min} is to enhance the effect of the warning coloration and thereby to avoid confusion with palatable species and to decrease the chance of mistaken attacks by potential predators (Brower et al., 1971; Turner, 1984; Guilford, 1986; Chai and Srygley, 1990). There are at least two possible ways to account for this function. First, mimetic females have conspicuous coloration on only their hindwings. However, because their hindwings partially overlap the forewings on the upper side, the downstroke of the hindwings should be synchronized with that of the forewings, and thus ϕ_{min} of the hindwings as well as the forewings is large. This large ϕ_{min}, or keeping the wing nearly horizontal, would enable their warning coloration to be readily recognized by predators flying above the butterflies. Second, we suppose that the large ϕ_{min} of mimetic females is related to the pattern of the flight path, because the up-and-down movement of a butterfly's flight path is related to the angular velocity, ϕ_{max}, and ϕ_{min}. The flight path would be large in cases of high angular velocity, large ϕ_{max}, and small ϕ_{min}. Thus, we predict that the flight path of non-mimetic females, which have small ϕ_{min}, will be more irregular than that of mimetic females. Chai and Srygley (1990) demonstrated that the flight paths of palatable butterflies with no defensive chemicals in their bodies were erratic (large up-and-down movement caused by wingbeats) to escape predators' attacks. By contrast, unpalatable butterflies flew regularly. This flight pattern may increase the conspicuousness of their warning coloration to enhance learning. Another possible function of the flight pattern in mimicry groups may be as a warning signal. Because flight pattern of butterflies with large ϕ_{min} and high WBF is so different compared to that of palatable butterflies, this flight pattern of the mimetic group per se may work as an effective warning signal.

For WBF, Φ, and angular velocity, similarity was also found between mimetic females and their unpalatable models, and between non-mimetic females and palatable butterflies, although the differences between these groups were not statistically significant, probably because of the conservativeness of the Bonferroni correction. Srygley (1999) and Srygley and Ellington (1999) confirmed that WBF converges within Müllerian mimicry groups, and that convergence of WBF may be the result of predators using WBF as a cue to distinguish among Müllerian mimicry groups. Our results suggest that there might be convergence of WBF also in Batesian mimicry groups. Srygley (2004) suggested that Batesian mimics and their models perform wingbeats with slow angular velocity to enhance the color signal. Similarly, in our study, mimetic females and their models tended to show slower angular velocity than palatable species/morphs. Therefore, this tendency may also serve to increase the efficiency of learning of the conspicuous coloration by predators and decrease the chance of mistaken attacks by them.

In males, we found significant differences between palatable butterflies and unpalatable species in almost all variables. Especially in ϕ_{min}, we detected significant differences between palatable and unpalatable species in both sexes. In Dudley's (1990) study in Papilioninae, *Papilio thoas*, which seems to be palatable because it feeds on species of Rutaceae, flew with small ϕ_{min} (–37°), whereas *Battus polydamas* and *Parides childrenae*, which are unpalatable (Srygley and Chai, 1990b), flew with large ϕ_{min} (–12° and –27°, respectively). These results suggest that in Papilioninae, large ϕ_{min} is a feature of unpalatable species, whereas small ϕ_{min} may be a feature of palatable species. However, because the three unpalatable species investigated so far (*Pachliopta aristolochiae*, *Battus polydamas*, and *Parides childrenae*) belong to the Troidini group, large ϕ_{min} may be a feature of the Troidini group and small ϕ_{min} may be characteristic of the Papilionini group. To determine whether interspecific differences in ϕ_{min} are caused by the ecological factors, we should study additional species.

In this study, we found behavioral differences in butterflies showing Batesian intraspecific polymorphism and behavioral similarity between mimetic females and their models. Although we did not detect significant differences in wing length between non-mimetic and mimetic females, it is premature to conclude that this similarity is caused solely by behavioral mimicry without any correlated morphological characteristics that enable the mimic to fly similarly to the model. Detailed morphological analysis is necessary to determine whether the behavioral similarity between models and Batesian mimics reflects "morphological similarity" or "intentional mimicry" (Chai and Srygley, 1990; Srygley and Chai, 1990a; Marden and Chai, 1991; Srygley and Dudley, 1993; Srygley, 1994).

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